Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of analytical chemistry

Candidate: Jaroslav Janák

Supervisor: RNDr. Hana Vlčková, Ph.D.

Title of Thesis: Development of MEPS-UHPLC-MS/MS methods for the determination of entecavir in kidney ultrafiltrate.

The thesis deals with the development and validation of sample preparation method for determination of entecavir in kidney ultrafiltrate, using techniques MEPS (microextraction by packed sorbent). The previously developed UHPLC-MS/MS method for the determination of entecavir in rat urine has been used for the analysis. Hydrophilic interaction chromatography by column Acquity BEH Amide and isocratic elution were employed. The composition of the mobile phase was acetonitrile and 5 mM ammonium acetate pH 4.0 in the ratio 75:25 (v:v). Entecavir $C_2{}^{13}N^{15}$ was used as the internal standard. Ionization of the analyte was carried out by electrospray in the positive ion mode and triple quadrupole was employed as the detector. Quantification of the analyte was realized by two SRM transitions of entecavir and internal standard method.

Based on the MEPS optimization method, porous graphitic carbon suitable for the analysis of polar compounds was selected as the most suitable sorbent. The mixture of acetonitrile and water in a ratio of 75:25 (v: v) as the optimal elution solvent and pure water as the washing solvent was chosen.

The method was validated. Linearity, precision, accuracy, selectivity and matrix effects were verified. The method was linear in the range 0.5 - 500 ng/ml and the limit of quantification was estimated at concentration of entecavir 0.5 ng/ml in a biological matrix. The precision was less than 4.5 % and the accuracy less than 106 %. Quantitative evaluation of matrix effects was carried out using post-extraction and values did not exceed 4 %. After validation of MEPS-UHPLC-MS/MS methods for the determination of entecavir in kidney ultrafiltrate, the method was applied to the series of real samples.

Keywords: Entecavir, microextraction by packed sorbent, UHPLC-MS/MS, method validation